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Activation of the CRF_1 receptor causes ERK1/2 mediated increase in GRK3 expression in CATH.a cells

Samina Salim, Brian Hite, Douglas C. Eikenburg*

Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204-5037, USA

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Abstract G-protein coupled receptor kinase 3 (GRK3) mediates desensitization of alpha$_2$-adrenergic (α_2-AR) and CRF_1 receptors. CRF_1 receptors, α_2-AR and GRK3, are localized to the primary source of noradrenergic inputs to higher brain centers critical in both the response to stress and the development of depression, namely, locus coeruleus (LC). This study utilizing CATH.a cells (derived from the LC), demonstrates for the first time, that the stress hormone, CRF selectively up-regulates GRK3 expression via an ERK1/2-mediated mechanism accompanied by the activation of Sp-1 and Ap-2 transcription factors. This observation has important implications for the regulation of stress signaling in the brain.

Keywords: Transcription; Nucleus; Protein synthesis; Kinase; Stress; Bipolar disorder

1. Introduction

The principal effectors of the stress response in the central nervous system (CNS), including corticotropin releasing factor (CRF), norepinephrine (NE) and epinephrine (EPI), bind to and activate G-protein coupled receptors (GPCRs) [1]. Prolonged receptor stimulation leads to desensitization via receptor phosphorylation by GPCR kinases (GRKs) causing receptor uncoupling from the G-protein and down-regulation via endocytosis of the receptor [2,3]. Receptors activated by NE and CRF, the α_2-adrenergic (AR) and CRF_1 receptors, respectively, are preferentially regulated by GRK3 [4–7]. This is in spite of the fact that GRK2 is present in higher concentrations within the cell.

Recent evidence from our laboratory demonstrates that, EPI, selectively up-regulates GRK3 expression and renders the $\alpha_{2A/B}$-AR more sensitive to agonist-stimulated desensitization and down-regulation in two neuronal cell models [6,7]. No changes in GRK2 expression were observed. Two other group of investigators have shown that CRF and kappa-opioid agonists selectively regulate GRK3 expression and that signaling through both the CRF_1 and kappa-opioid receptors is preferentially regulated by GRK3 and not GRK2 [4,5,8]. Most recently, we have observed that EPI treatment, in both human neuroblastoma BE(2)-C and rodent neuroblastoma–glioma BN17 cells, increases GRK3 expression while GRK2 expression is unaltered [9]. Hence, GRK3, previously considered to be subservient to its related kinase, GRK2 with no specialized functions, is now beginning to be appreciated as a novel kinase with specialized functions in several important systems including CNS.

Within the CNS, maintaining appropriate responsiveness to the principal effectors of the stress response system including CRF, NE and EPI is critical in several neuropsychiatric disorders including depression, bipolar disorder and schizophrenia to name a few [10–14]. Stress is associated with activation of noradrenergic neurons both centrally and peripherally. Within the brain, the noradrenergic projections to the hypothalamus, amygdala and cortex are the noradrenergic neurons originating in locus coeruleus (LC) [1,15–17]. CRF_1 receptors are located in LC or its dendritic fields and CRF stimulates LC firing [1,15,16]. In addition there are α_{2A}-AR in LC that inhibit the firing of LC neurons when stimulated [17,1,15]. Moreover, LC is enriched with GRK3, highlighting the potential for an important regulation of CRF_1 and α_{2A}-AR signaling by GRK3 in LC. Hence, GRK3 and the regulation of its expression may play a significant role in the regulation of both CRF_1 and α_2-AR signaling in LC neurons.

Based upon our recent observations of selective increase in GRK3 expression in two neuronal models in response to EPI treatment and the potential for an important role of GRK3 in the regulation of CRF_1 and α_{2A}-AR signaling in LC, the present study was undertaken employing a neuronal cell model derived from LC, CATH.a cells. The objective of the present study was to provide additional evidence that another mediator of the stress response, namely CRF, selectively activates GRK3 transcription and protein upregulation in a manner consistent with an important role for GRK3 in modulating the response to stress within the CNS.

*Corresponding author. Fax: +1 713 743 1884.
E-mail address: deikenburg@uh.edu (D.C. Eikenburg).

Abbreviations: GRK, G-protein coupled receptor kinases; EPI, epinephrine; AR, adrenoceptor; PVDF, polyvinylidene difluoride; GPCRs, G-protein coupled receptors; RT-PCR, reverse transcriptase polymerase chain reaction; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase

2. Materials and methods

2.1. Material

The following were purchased from the indicated sources:

(–) Epinephrine bitartrate (EPI), phenylmethylsulphonylflouride (PMSF), MEK-inhibitor (U0126), (Sigma Chemical Co., St. Louis, MO); fetal bovine serum and penicillin-streptomycin (Atlanta

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Biological, Norcross, GA). RNeasy Mini kit (cat# 74104) was purchased from Qiagen and Superscript one step reverse transcriptase polymerase chain reaction (RT-PCR) kit (cat# 11922-028) from Invitrogen life technologies. Ethidium bromide (cat# H5041) and Loading dye (cat# G190A) from Promega Corp. Madison, WI. One hundred base pair molecular ruler (cat# 170-8202) and pre-stained SDS–PAGE protein marker (cat# 161-0324) were from Biorad. Anti-Sp-1 (sc-59), Ap-2 (sc-184) antibodies and horse-radish peroxidase conjugated anti-rabbit secondary antibody were purchased from Santa Cruz Biotechnology Inc. Santa Cruz, CA. Oligofeactamine reagent (#12252-011) was purchased from Invitrogen Life Technologies and OPTI-MEM I (#31985-070) was from GIBCO. Sp-1 (#sc-29488) and Ap-2 (sc-29697) siRNA was from Santa Cruz Biotechnology Inc.

2.2. Cell culture

The CATH.a cells were maintained in RPMI-1640 medium supplemented with 4% fetal calf serum, 8% horse serum, penicillin, streptomycin. The cells were maintained in a humidified atmosphere (5% CO_2:95% air). All cells were grown until 60–70% confluent prior to CRF treatment throughout the study. CATH.a cells natively express α_{2A}-AR, CRF [18,19], GRK2 as well as GRK3.

2.3. Pre-treatment

CATH.a cells were pre-treated with vehicle (medium containing 0.1 mM Ascorbate and 1 μM Sodium metabisulphite), or vehicle containing 10 nM–10 μM CRF for 24 h for protein expression measurement and 45 min for mRNA expression measurement.

2.4. siRNA transfection

CATH.a cells were plated on six-well dishes and transfected with siRNA when ~60% confluent. Before transfection, the cells were incubated with serum and antibiotic-free RPMI-1640 media. siRNA duplex (0.1, 0.5 or 1 μM) was incubated with oligofectamine transfection reagent (0.6% in OPTI-MEM I medium) at room temperature for 10 min. The complex was then added to the cells and incubated at 37 °C with 5% CO_2. After 6 h, serum and antibiotic were added to the media. After 24 h of transfection, the cells were washed once with PBS and harvested in 250 μl hypotonic lysis buffer (50 mM Tris–HCl, pH 7.5, 4 mM EDTA plus protease inhibitors), lysed and subjected to SDS–PAGE as described below.

2.5. MEK1/2 inhibitor (U0126) and chelerythrine chloride (CC) treatment

Cells when ~70% confluent were treated in RPMI-1640 media for 2 h in the presence of 10 μM MEK1/2-inhibitor (U0126) or CC at 37 °C incubator at 5% CO_2:95% air. The cells pre-treated with the inhibitors were subsequently treated with vehicle/CRF for the desired times.

2.6. Western blot analysis

Cells were washed once with 1X PBS buffer (pH 7.4), lysed immediately in 100–200 μl of hypotonic lysis buffer (50 mM Tris–HCl pH 7.4, 4 mM EDTA, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin and 1 μg/ml pepstatin) followed by 5–6 passes through a 23-gauge needle and subsequently centrifuged at 1000 rpm for 10 min to remove cellular debris and nuclei. The lysates thus obtained were checked for their protein concentration using Pierce's protein detection kit [20]. The cell lysates were diluted with 4× Laemmli buffer (50 mM Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 0.1 mg/ml bromophenol blue) and resolved on SDS–PAGE (10% gel) and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Pharmacia Biotech, Buckinghamshire, England) and levels of GRK3, GRK2, GAPDH, phospho-specific ERK1/2, total ERK1/2, Sp-1 and Ap-2 proteins were determined by immunoblotting using antibodies as indicated. The immunoreactive bands were detected by a horse-radish peroxidase-conjugated secondary antibody and the blots were developed using chemiluminescence reagent made by adding *p*-coumarric acid and luminol (Sigma–Aldrich) in 1 M Tris–HCl and hydrogen peroxide solution. Chemiluminescence was detected by an Alpha Innotech imaging system and densitometry was quantified using Fluorchem FC8800 software.

2.7. Protein estimation

Protein concentrations were determined by the Pierce protein detection kit (Pierce, Rockford, IL Cat# 232009) using BCA protein assay reagent A (Cat# 23223) and reagent B (Cat# 23224) [20].

2.8. RT-PCR measurement

Total RNA was isolated from CATH.a cells after vehicle/CRF treatment for 45 min using the RNeasy RNA isolation kit as per manufacturer's guidelines. For each RT-PCR reaction 150 ng of total RNA was used and the purity of the RNA was determined by the ratio of the readings on the spectrophotometer at 260 nm and 280 nm. The RNA used for RT-PCR had a ratio of 1.5–2.0. RT-PCR was carried out in a programmable thermal controller (iCycler, Bio-Rad) with the following oligonucleotide primers:

(1) Forward primer for detecting GRK3: 5′-AATTGAGGCCAGGAAGAAGGCTA-3′ which corresponds to position 1605–1627 rat GRK3 cDNA [21].
(2) Reverse primer for detecting GRK3: 5′-TCAGAGGCCGCTGCTATTTCTGTGACA-3′ which corresponds to position 2041–2067 rat GRK3 cDNA [21].
(3) Forward primer for detecting GRK2 was: 5′-GTTGCTGCAGAGGGATGTCAACCG-3′ which corresponds to position 1388–1411 of human GRK2 cDNA [5].
(4) Reverse primer for detecting GRK2 was: 5′-GTCAGAAAGGGGTTGCCCATCTTGG-3′ which corresponds to position 1828–1804 of human GRK2 cDNA [5].
(5) Forward primer for detecting rat GAPDH was: 5′-TACTCCTTGGAGGCCATGTA-3 [22].
(6) Reverse primer for detecting rat GAPDH was: 5′-CGTGGAGTCTACTGGCGTCT-3′ [22].

Both cDNA synthesis and PCR were performed using superscript RT-PCR kit in a single tube employing gene-specific primers and total RNA isolated from CATH.a cells using the RNeasy minikit. One cycle of 50 °C for 30 min and 94 °C for 2 min was carried out for cDNA synthesis and pre-denaturation, followed by 40 cycles of 94 °C for 15 s (denature), 55 °C for 30 s (anneal) and 72 °C for 10 min for extension. PCR products were separated by electrophoresis through an agarose gel (2%) and visualized by ethidium bromide staining.

2.9. Immunofluorescence

The cells were processed for immunofluorescence as described previously [23]. Briefly, CATH.a cells were grown on poly-D-lysine coated 20 × 20 mm glass cover slips to 40–70% confluence. The cells were then exposed to vehicle/CRF (10 μM) for 15 min. Next, the cells were washed with PBS containing 1.2% sucrose (PBSS) and fixed with 4% paraformaldehyde in PBSS at 4 °C for 15 min. The following steps were carried out at room temperature. The fixed cells were incubated in 0.034% L-lysine, 0.05% Na-meta-periodate for 20 min, washed and permeabilized with 0.2% Triton X-100 for 10 min. After further wash, the cells were blocked with 10% normal goat serum for 15 min. Primary (anti-Sp-1 or anti-Ap-2 antibody) and secondary antibody (anti-rabbit cy3-conjugated antibody) were diluted in PBSS with 0.2% goat serum and 0.05% Triton X-100. The cells were incubated with anti-Sp-1 or anti-Ap-2 antibody for 1 h at room temperature or overnight at 4 °C, followed by cy3-conjugated secondary antibody for 1 h in complete darkness. The cells were washed three times with PBSS before and after incubation with secondary antibody. The cover slips were then mounted on slides with a drop of a 1:1 mixture of Mowiol solution and VECTASHIELD mounting medium with DAPI. Fluorescence examination of at least 5–6 fields on the same slide was performed under an oil immersion objective (×60, 1.4 NA) using a filter selective for cy3 or DAPI using an Olympus IX81 Fluorescence Deconvolution Microscope System. DAPI staining enabled us to determine the area occupied by the nucleus in the CATH.a cells in order for us to monitor movement of Sp-1 and/or Ap-2 into the nucleus from the cytosol. At each time point a representative group of cells were assessed for the extent of nuclear translocation. As a negative control we stained the cells either with primary (anti-Sp-1/Ap-2) or secondary (anti-rabbit cy3 conjugated) antibody alone in order to determine the specificity of the fluorescence signal. Images were optimized using AutoDeblur and Autovisualization deconvolution software and transferred to Adobe Photoshop 5.5 for the production of final figures.

2.10. Data analysis

Data are expressed as means ± S.E.M. Comparisons between groups were made either by Student's *t*-test or one way ANOVA followed by Tukey's post hoc test where appropriate (GraphPad Software Inc., San Diego, CA), and groups were considered significantly different if $P < 0.05$.

3. Results

The effect of CRF treatment on the level of GRK3 and GRK2 protein expression was determined in CATH.a cells. CATH.a cells endogenously express α_{2A}-AR and CRF_1 receptors and also express GRK3 and GRK2. CATH.a cells were incubated with the MEK1/2 inhibitor, U0126 (10 μM; 2 h) prior to chronic (24 h) exposure to 10 μM CRF. Chronic CRF treatment (10 μM) significantly increased cellular GRK3 protein expression (Fig. 1A) with no effect on GRK2 protein expression (Fig. 1B) compared to the respective vehicle control, $P < 0.05$, $n = 3$. The internal protein loading control, GAPDH, remained unaltered. Pre-incubation of CATH.a. cells with the MEK1/2 inhibitor, U0126 (10 μM; 2 h) prior to

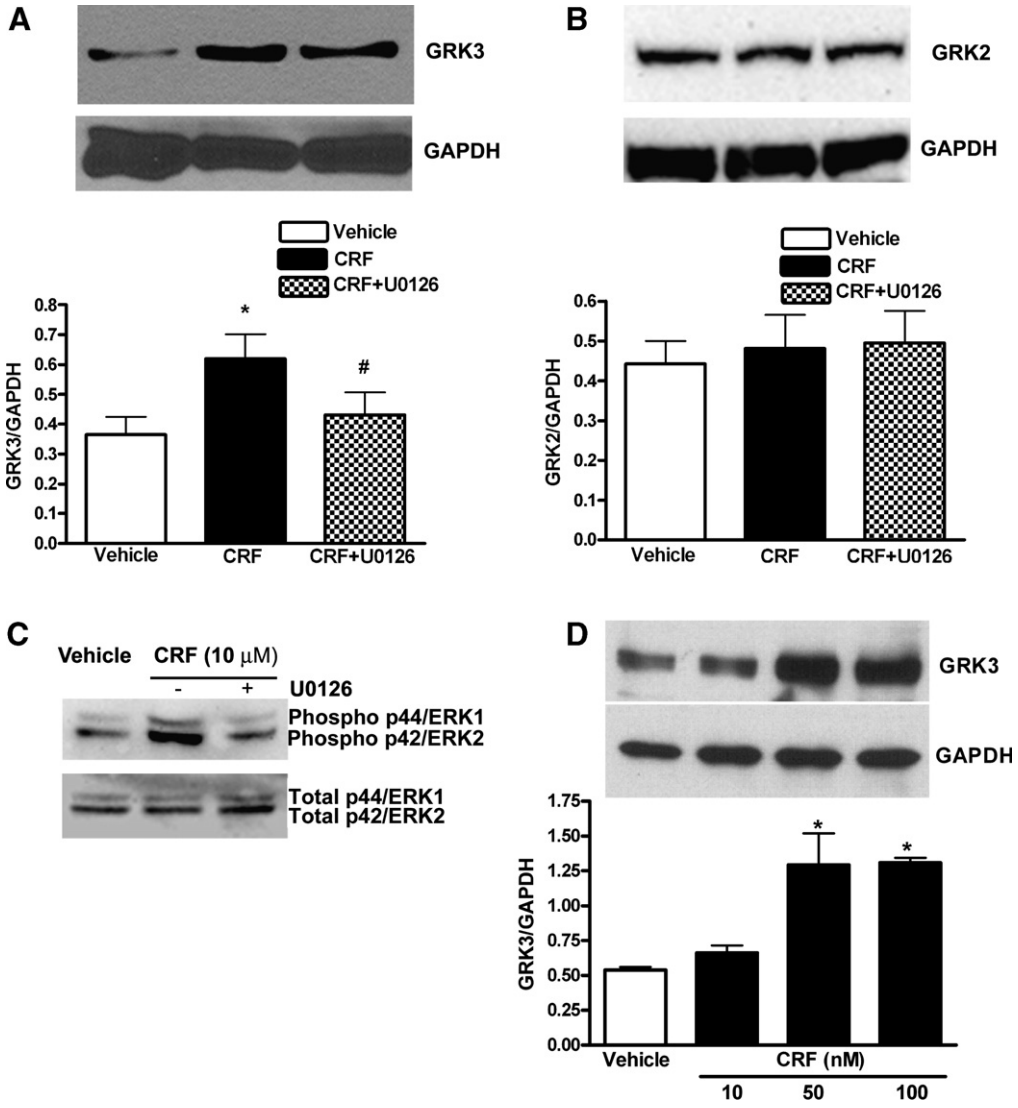

Fig. 1. Involvement of the ERK1/2 pathway in the up-regulation of GRK3 protein expression in CATH.a cells pre-treated with 10 μM CRF. Cellular levels of GRK3 (A) GRK2 (B) Phospho-ERK1/2 and Total ERK1/2 (C) were determined by Western blot analysis in CATH.a cells pre-treated with vehicle or 10 μM CRF for 24 h. The effect of 24 h CRF treatment on GRK3 protein expression also was determined at 10, 50 and 100 nM concentrations of CRF in CATH.a cells (D). The cells were washed with PBS, harvested in hypotonic lysis buffer, subjected to SDS–PAGE and probed with anti-GRK3 and GRK2 antibody, respectively. The levels of GAPDH protein, used as an internal loading control, were determined by stripping the same blot and re-probing with anti-GAPDH antibody. The cellular levels of GRK3 are significantly increased (*) following 24 h of pre-treatment with 10 μM CRF as compared to the vehicle control, $P < 0.05$, $n = 3$ (A). The levels of GRK2 remained unchanged following 24 h of pre-treatment with 10 μM CRF as compared to the vehicle control (B). CATH.a cells were treated with 10 μM CRF for 15 min, the cells harvested as above, equal amounts of protein subjected to SDS–PAGE and probed with anti-phospho-ERK1/2 and total ERK1/2 antibody, respectively. The levels of total ERK1/2 protein were determined by stripping the same blot and re-probing with anti-total ERK1/2 antibody. The cellular levels of phospho-ERK1/2 are significantly increased (*) following 15 min of pre-treatment with 10 μM CRF as compared to the vehicle control, $P < 0.05$, $n = 3$ (C). The effect of 24 h CRF treatment on GRK3 was concentration-dependent with a threshold between 10 and 50 nM (D). Panels A–D show representative immunoblots of at least three different experiments and the relative levels of GRK3, GRK2, phospho-ERK1/2 and total ERK1/2 protein were quantified by densitometry on an Alpha Innotech imaging system using FC8800 software.

CRF treatment prevented the increase in GRK3 protein up-regulation (Fig. 1A) while GRK2 protein expression remained unchanged (Fig. 1B). Incubation of CATH.a cells with 10 μM CRF for 15 min increases ERK1/2 phosphorylation (Fig. 1C), indicating ERK1/2 activation, and pre-treatment with U0126 (10 μM, 2 h) prevented this increase in ERK1/2 phosphorylation (Fig. 1C). MEK1/2 inhibitor treatment by itself had no effect on GRK3 or GRK2 protein expression (data not shown). The effect of 24 h CRF treatment on GRK3 was concentration-dependent with a threshold between 10 nM and 50 nM (Fig. 1D).

Treatment with 10 μM CRF also significantly increased GRK3 mRNA expression as compared to the vehicle control, $P < 0.05$, $n = 3$, within 45 min of CRF treatment (Fig. 2A) while GRK2 mRNA expression remained unchanged (Fig. 2B). GAPDH, used as a DNA amplification control, remained unchanged. Preincubation of the cells with the MEK1/2 inhibitor, U0126 (10 μM; 2 h) prior to CRF treatment prevented the increase in GRK3 mRNA. Similar to protein, the effect of CRF on GRK3 mRNA was concentration-dependent, with a threshold of 10 nM (Fig. 2C).

Since previous reports indicate that GRK2 expression is regulated by PKC [24], we also examined the effect of CRF treatment on GRK3 mRNA in the presence of the PKC inhibitor, chelerythrine chloride. The increase in GRK3 mRNA in response to CRF (10 μM) treatment was unaffected by pre-treatment of CATH.a cells with chelerythrine chloride (10 μM) (Fig. 3).

Nuclear translocation of Sp-1 and Ap-2, two transcription factors activated by ERK1/2, also increased in response to CRF treatment (Fig. 4A and B). CATH.a cells were treated

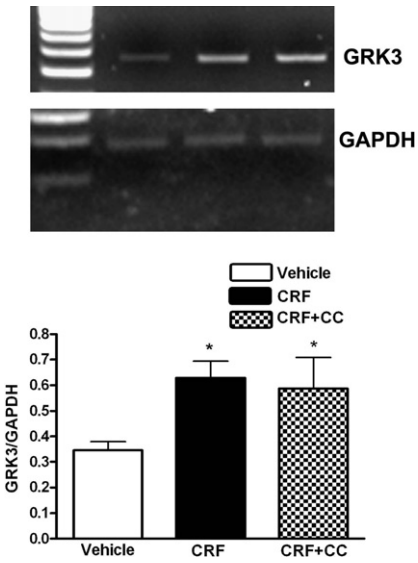

Fig. 3. Quantitation of mRNA levels for GRK3 in CATH.a cells exposed to CRF (10 μM) treatment in the presence or absence of chelethrine chloride. CATH.a cells were pre-treated with 10 μM CC in the absence of serum for 2 h and then subsequently treated with vehicle/CRF (10 μM) for 45 min. Total RNA was isolated from CATH.a cells and reverse transcribed and then amplified using primers for GRK3 and GAPDH. PCR products were electrophoresed through a 2% agarose gel, stained with 0.5 μg/ml ethidium bromide, and visualized under UV light. 100–3000 bp molecular ruler indicated on the left-hand side of the gel is used for reference. Shown is the representative RT-PCR gel depicting GRK3 mRNA expression. Each bar represents means ± S.E.M. values for the densitometric quantitation ratio of GRK3 mRNA normalized to the GAPDH mRNA (DNA amplification control) obtained from three independent experiments.

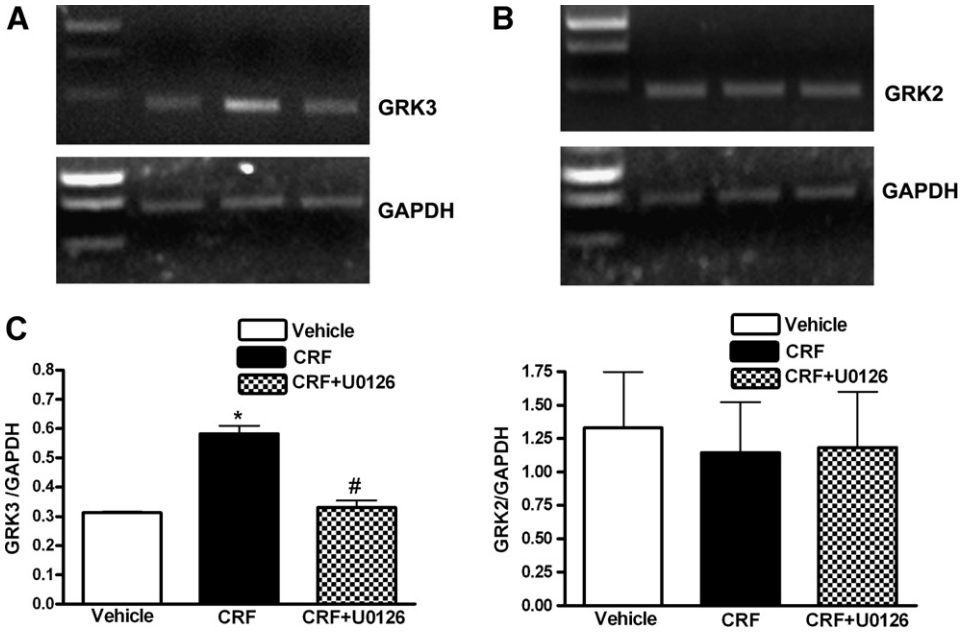

Fig. 2. Quantitation of mRNA levels for GRK3, GRK2 in CATH.a cells exposed to CRF (10 μM) treatment. CATH.a cells were pre-treated with the MEK1/2 inhibitor, U0126 (10 μM) in the absence of serum for 2 h and then subsequently treated with vehicle /CRF (10 μM) for 45 min. Total RNA was isolated from CATH.a cells, reverse transcribed and then amplified using primers for GRK3 (A, C), GRK2 (B). PCR products were electrophoresed through a 2% agarose gel, stained with 0.5 μg/ml ethidium bromide, and visualized under UV light. A 100–3000 bp molecular ruler indicated on the left-hand side of the gel is used for reference. (*) denotes significantly different from vehicle at $P < 0.05$; (#) significantly different from CRF at $P < 0.05$. The effect of CRF on GRK3 mRNA was concentration-dependent, with a threshold of 10 nM (C). Shown is the representative RT-PCR gel depicting up-regulation of GRK3 mRNA expression. Each bar represents means ± S.E.M. values for the densitometric quantitation ratio of GRK3 or GRK2 mRNA normalized to the GAPDH mRNA (DNA amplification control) obtained from three independent experiments.

with CRF (10 μM) or vehicle for 15 min and then processed for immunofluorescence as described previously [23]. In vehicle-treated cells, Sp-1 (red signal) was dispersed throughout the cytosol with almost no nuclear localization (blue signal) (Fig. 4A). In cells treated with CRF, the intensity of the Sp-1 signal in the nuclei increased dramatically, staining the entire nucleus red. Moreover, as nuclear localization of Sp-1 increased, the Sp-1 signal disappeared from the cytosol. Similar results were obtained for the transcription factor, Ap-2, with significantly increased nuclear localization in response to CRF treatment (Fig. 4B). These results are similar to those recently obtained in two neuronal cell lines, BE(2)-C and BN17, in response to EPI treatment [9]. In addition, we investigated the direct involvement of Sp-1 and Ap-2 in regulation of GRK3 protein expression (Fig. 5A–C). Transfection of CATHa. cells with 0.1, 0.5 or 1 μM Sp-1 siRNA or Ap-2 siRNA for 24 h reduced the expression of Sp-1 and Ap-2 transcription factors. Treatment with 1 μM Sp-1 si RNA or Ap-2 siRNA reduced Sp-1 and Ap-2 protein expression levels ~50%. GAPDH was used as an internal loading control and remained unaltered (Fig. 5A and B). Pre-treatment with 1 μM Sp-1 siRNA, 1 μM Ap-2 siRNA or the combination, significantly reduced the CRF-mediated increase in GRK3 protein expression. siRNA oligos were added 6 h before the addition of CRF (10 μM, 24 h) and remained present throughout the CRF treatment (Fig. 5C).

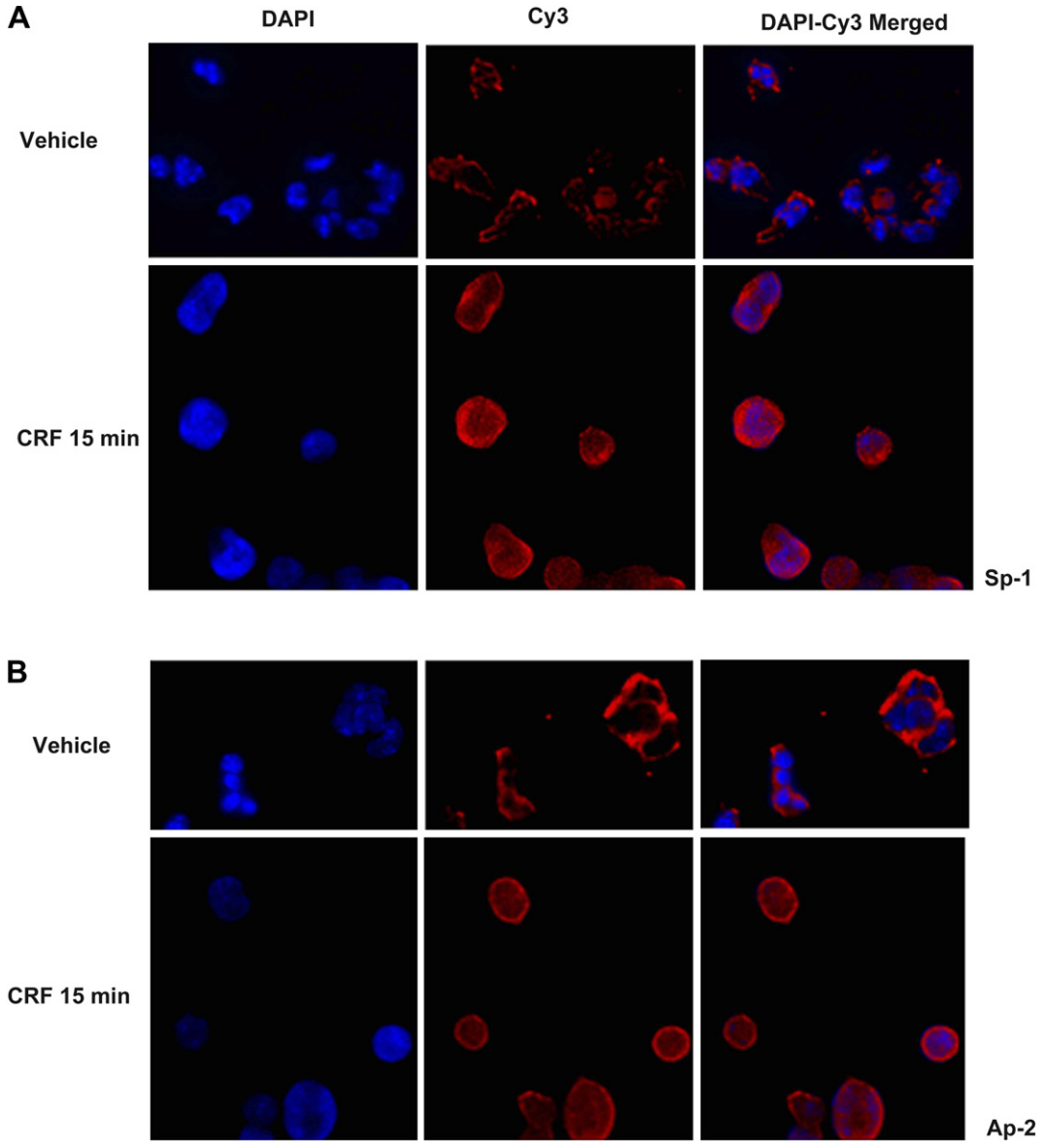

Fig. 4. CRF treatment causes nuclear translocation of Sp-1 and Ap-2 in CATH.a cells: Cells were treated with CRF (10 μM)/vehicle for 15 min and processed for immunofluorescence as described in detail in Section 2. Samples were incubated with rabbit anti-Sp-1/Ap-2 antibody followed by addition of anti-rabbit cy-3. Cells also were stained with DAPI for visualization of cell nuclei. Shown are representative images and overlays of at least 5–6 fields on the same slide of Sp-1/Ap-2 (visualized by selective cy3 filter, red), DAPI (staining for cell nuclei visualized by selective DAPI filter, blue) and overlay of the two images under an oil immersion objective (×60, 1.4NA) on an Olympus 1X81 Fluorescence Deconvolution Microscope System.

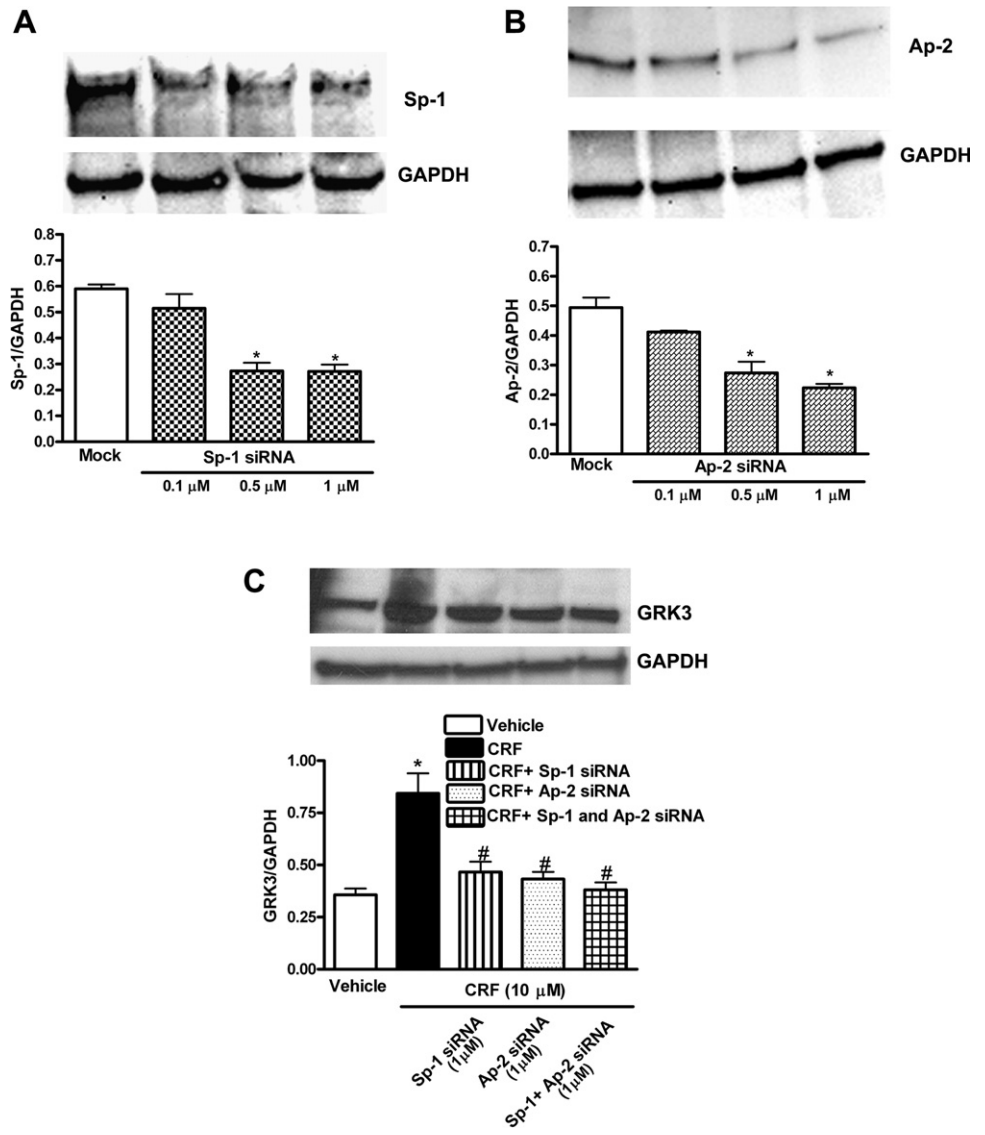

Fig. 5. Sp-1 or Ap-2 siRNA treatment prevents CRF-mediated GRK3 protein up-regulation in CATH.a cells. Cells were transfected with 0.1 μM, 0.5 μM and 1 μM Sp-1 (A) or Ap-2 (B) siRNA for 24 h in OPTI-MEM medium. The cells were washed with PBS, harvested in hypotonic lysis buffer, subjected to SDS–PAGE and probed with anti-Sp-1 or Ap-2 antibody, respectively. The levels of GAPDH protein used as an internal loading control were determined by stripping the same blot and re-probing with anti-GAPDH antibody. The cellular levels of Sp-1 or Ap-2 are significantly decreased (*) following 24 h of pre-treatment with 0.5 or 1 μM Sp-1 or Ap-2 siRNA as compared to the vehicle control, $P < 0.05$, $n = 3$. (C) CATH.a cells were pre-treated with 1 μM Sp-1 siRNA, 1 μM Ap-2 siRNA, or the combination. siRNA oligos were added 6 h before the addition of CRF (10 μM, 24 h) and remained present throughout the CRF treatment. CRF treatment significantly increased (*) GRK3 protein expression and pre-treatment with 1 μM Sp-1 or Ap-2 siRNA significantly reduced (#) the CRF-mediated increase in GRK3 protein expression, $P < 0.05$, $n = 3$.

4. Discussion

The results of the present study demonstrate that another stress hormone/neurotransmitter, CRF, causes a selective increase in GRK3 expression via an ERK1/2 dependent mechanism. In addition, this increase in GRK3 mRNA and protein is accompanied by increased nuclear localization of the transcription factors Sp-1 and Ap-2. Moreover, siRNA treatment directed against Sp-1 and Ap-2 largely prevented the CRF-mediated increase in GRK3 expression. These effects were observed in CATH.a cells, a cell line derived from the mouse LC, a region of the brain which plays an important role in the CNS response to stress.

A selective increase in GRK3 mRNA in response to CRF has been previously reported. CRF increased GRK3 mRNA in Y79 retinoblastoma cells [5] with no change in GRK2 mRNA. These investigators observed a similar response to the pituitary adenylyl cyclase-activating polypeptide (PACAP) receptor type 1 (PAC1) agonist, PACAP38 [25]. Several other reports of selective increases in GRK3 mRNA have also been reported [26,27]. Our finding of an ERK1/2-dependent and selective increase in GRK3 expression in CATH.a cells via activation of transcription factors (Sp-1 and Ap-2) that are postulated to bind to the putative promoter region of GRK3 gene implies that the ERK1/2-dependent regulation of GRK3 expression recently observed in BE(2)-C and BN17 cells [9], extends to other important neuronal cell models. Moreover, the CATH.a cell model (derived from the LC region of the brain) utilized in the present investigation is particularly important for the following reasons. *First*, CRF_1 receptors

are located in LC and CRF stimulates LC firing [1,15,16]. *Second*, α_{2A}-AR also are present in LC and inhibit LC firing when stimulated [17,1,15]. *Third*, signaling for both receptors is preferentially regulated by GRK3 [4–6]. Finally, it is well documented that stressors activate the LC, a region of the brain that is fundamentally important in the response to stress, depression and other neuropsychiatric disorders [17,1,15,16] and controls both the endocrine and noradrenergic function [17,15,28]. This brings into focus the potential for an important regulation of CRF_1 and α_{2A}-AR signaling by GRK3 in LC.

The regulation of GRK3 expression may have important implications in stress and stress-associated affective disorders such as bipolar disorder (BPD). A mutation in the putative promoter region of the human GRK3 gene has already been identified in a sub-population of BPD patients, suggesting an interesting link between BPD and GRK3 [29]. Two neurotransmitters/hormones important in stress, namely, EPI and CRF, both signal through receptors preferentially regulated by GRK3. Therefore, one can envision a regulatory mechanism whereby chronic stimulation by EPI or CRF in stress results in up-regulation of GRK3 expression, which then renders the receptors more sensitive to desensitization to protect against over-stimulation both within the brain and at peripheral target organs.

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